DNA Looping Mediated Transcriptional Regulation

LUN CUI

A thesis submitted for the degree of Doctor of Philosophy

Discipline of Biochemistry
School of Molecular and Biomedical Science
The University of Adelaide
December 2013
Contents

Abstract

Declaration

List of Publications

Acknowledgements

Chapter 1: Introduction
  DNA looping and transcriptional regulation
  DNA looping mediated transcriptional regulation in phage lambda
  Measuring the efficiency of long-range DNA looping
  Synthetic biology tools for DNA integration into bacterial chromosome

Chapter 2: Enhancer-like long-range transcriptional activation by λ CI-mediated DNA looping

Chapter 3: Bacteriophage λ repressor mediates the formation of a complex enhancer-like structure

Chapter 4: Quantitation of DNA tethering in long-range DNA looping in vivo and in vitro using the Lac and λ repressor

Chapter 5: One-step cloning and chromosomal integration of DNA

Chapter 6: Repurposing site-specific recombinases for synthetic biology

Chapter 7: Conclusions and Future Directions
Chapter 8: References for chapters 1 and 6
Abstract

Protein binding to DNA sequences is the foundation of transcriptional regulation. By binding at specific DNA sequences, such as promoters, proteins can recruit other proteins to regulate gene transcription. Some proteins, which bind at separated DNA binding sites, can interact via the formation of a DNA loop. DNA looping is essential in many processes, such as replication, recombination and gene regulation. In prokaryotic systems, DNA looping is involved in some genetic switches, which can control bacteriophage lysogenic/lytic pathways or bacterial catabolic pathways. Small DNA loops are essential for effective repression of several operons in bacteria. In eukaryotic systems, enhancers are distal gene regulatory elements, which can be located far away from the promoter. The formation of DNA looping is thought to be necessary for the function of enhancers.

Bacteriophage λ CI repressor activates the transcription of its own gene, while the CI mediated looping represses its own transcription. This DNA looping mediated long-range repression improves the efficiency of the lambda lysogenic/lytic switch. However, evidence in the literature suggests an additional activation effect of λ CI DNA looping. In Chapters 2 and 3, I investigated this long-range λ CI DNA looping mediated transcriptional activation. By using a synthetic λ CI DNA looping reporter system, I confirmed that λ CI DNA looping can mediate enhancer-like long-range transcriptional activation. In vivo experiments showed that the λ PRM promoter was activated by the α C-terminal domain (CTD) of RNA polymerase contacting an UP element located 2.3 kilobases away from the PRM promoter. A physicochemical model of the in vivo data showed that an RNA polymerase α subunit recruitment mechanism could fully explain this activation effect. DNA-protein structural modelling found that the bending of linker sequence between OL2 and the UP element is required for the contact.

The efficiency of long range DNA looping has been studied in Chapter 4. In vivo Lac looping and lambda CI DNA looping constructs were used to generate data for calculating DNA looping efficiency. DNA loop sizes ranging
from 250 bp to 10000 bp were tested. Tethered particle motion (TPM) experiments, performed by our collaborators, generated in vitro DNA looping data by using Lac mediated DNA loops ranging from 600 bp to 3200 bp. Based on these in vitro and in vitro data, mathematical modelling calculated DNA looping parameters for understanding DNA looping efficiency.

The insertion of DNA looping constructs into the *E.coli* chromosome (by using a bacteriophage integrase based approach) also led us to make a one-step integration system (OSIP), described in Chapter 5. The OSIP system is a set of OSIP plasmids, which can mediate one-step bacterial chromosomal integration of DNA sequences. The cloning module of each OSIP plasmid has both the integrase gene and corresponding att sequences, which are required for integrating the OSIP plasmid into the bacterial chromosome. An integration protocol, called clonetegration, was developed by coupling the OSIP system with in vitro isothermal DNA assembly. Clonetegration bypasses plasmid propagation and purification procedures by transferring assembly products directly into target competent cells.
Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to LUN CUI and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed __                                      Date __05/03/2014__
List of Publications


Acknowledgements

I would like to express my sincere gratitude to the following people:

- Dr. Keith Shearwin, for supervision and guidance, and for giving me opportunity and support to do this PhD.
- Dr. Ian Dodd, for supervision and guidance, and for giving me opportunity and support to do this PhD.
- Prof. Barry Egan, for support on this PhD, and help on my research proposal.
- Miss Ranran He, my friend, for inspiring and improving my spiritual momentum, for bringing me endless happiness, and for support on this PhD.
- Dr Francois St-Pierre, my collaborator in Stanford University, for help in the OSIP project, and suggestions on finding postdoctoral position.
- Dr. Iain Murchland, David Priest, Julian Pietsch, Dr. Andrew Hao, Dr. Linda Shearwin, Kaitlyn Tepper, Dr. Alex Ahlgren-Berg, Erin Cutts, for support during this PhD.
- Dr. Yuqi Fan, my old friend, for support on this PhD.
- Yun Luo, my older sister, for supporting me to study abroad. She died in a car accident in 2010. Here I express my deep sorry for her loss. You are always in my thoughts and prayers.
- Prof. Lianfu Deng, supervisor of my Masters degree in Ruijin Hospital, for supporting me to study abroad.

I would like to express my sincere thanks and appreciation to the following families:

- My parents (Zusheng Cui, Cuilan Yu) and my old sister (Ping Cui) have made great sacrifice to support my study in China and in Australia. My parents support me on everything. My old sister has done a lot for me.
- My uncle (Xueming Yu)'s family, for support and help on my family.
• Ranran He’s parents (Prof. Xiaoyang He, Huijun Zhou), for support on me, and for help on my health.

I would also like to express my thanks to the following organizations:

• Shanghai Institute of Traumatology and Orthopaedics (for supporting me to study abroad)
• Ruijin Hospital, Shanghai, China (for supporting me to study abroad)
• Shanghai Jiao Tong University, School of Medicine (for providing me opportunity to study abroad)
• University of Adelaide (for providing me opportunity to do this PhD)
• China Scholarship Council (for providing me scholarship for this PhD)
• Human Frontiers Scientific Program (for providing funding for research on this PhD)
• Australian Research Council (for providing funding for research on this PhD)